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Effects of Hyperthermal Stress on the Ultrastructure of Platelets With Reference to the Localization of Platelet Peroxidase and Fibrinogen In Vivo

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Ultrastructure of platelets with the localization of platelet peroxidase and fibrinogen through 3-min 47°C hot-spring bathing was investigated in eight healthy volunteers. The mean sublingual temperature rose about 1.8°C 5 min after the start of bathing. The frequencies of fold, pseudopods, vacuoles, and centralization were increased after bathing. Platelet peroxidase activity was decreased after bathing. Furthermore, fibrinogen was decreased in α-granules after bathing. Thus, hyperthermal stress in vivo may activate platelets, resulting in consumption of platelet peroxidase and fibrinogen. Am. J. Hematol. 56:244–247, 1997.

Key words: platelet; ultrastructure; hot-spring bathing; thrombosis

INTRODUCTION

Coagulopathy is recognized in heat stroke occurring among pilgrims in the summer and in whole-body hyperthermia for the treatment of neoplasms [1,2]. We have noted that thrombotic diseases often occur in people after bathing in very hot hot-springs [3]. Bathing in hot hot-spring water is a popular custom in Japan and people enjoy such bathing as a leisure activity [4,5]. We previously reported that such very hot hot-spring bathing decreased fibrinolytic capacity [6]. White described that platelet shape was changed by in vitro heating [7]. Thus, to further clarify the mechanism of the onset of thrombotic events after very hot hot-spring bathing, we examined the effects of hyperthermal stress on platelet morphology with reference to the localization of platelet peroxidase (PPO) and fibrinogen. PPO is detected in the dark tubular system and is associated with the synthesis of prostaglandins [8]. Fibrinogen is reserved in α-granules of platelets and is released by stimulation. Therefore, PPO and fibrinogen are considered good indicators for estimating platelet activation.

MATERIALS AND METHODS

Effects of Very Hot Hot-Spring Bathing on the Platelets

Eight healthy male volunteers aged 22 to 35 years participated in this study after providing informed consent. Their body mass indices ranged between 19.1 and 22.1 kg/m^2. They had taken no medication for at least 10 days and refrained from smoking and drinking tea, coffee, and alcohol overnight. Only water was allowed to be consumed throughout each experiment. After an hour's relaxation in a comfortable room, they took a 3-min 47°C hot-spring bath at 1:30 P.M. Subjects squatted with bent knees, soaking in water up to their chins in a bathtub about 1 m deep filled with 47°C hot-spring water for exactly 3 min. Then, they spent free time in a comfortable room until the end of the experiment. The method of taking the 3-min 47°C hot-spring bath has been passed down for over 130 years at Kusatsu-spa and the 3-min time is the limit for which one can bear such very hot water [4,5].

Before and at 15 and 180 min after the start of bathing, venous blood was carefully collected without a tourniquet into a plastic syringe containing acid citrate dextrose solution. The blood sample was gently transferred to a plastic tube and centrifuged to obtain platelet-rich plasma. The platelet-rich plasma was transferred to an-

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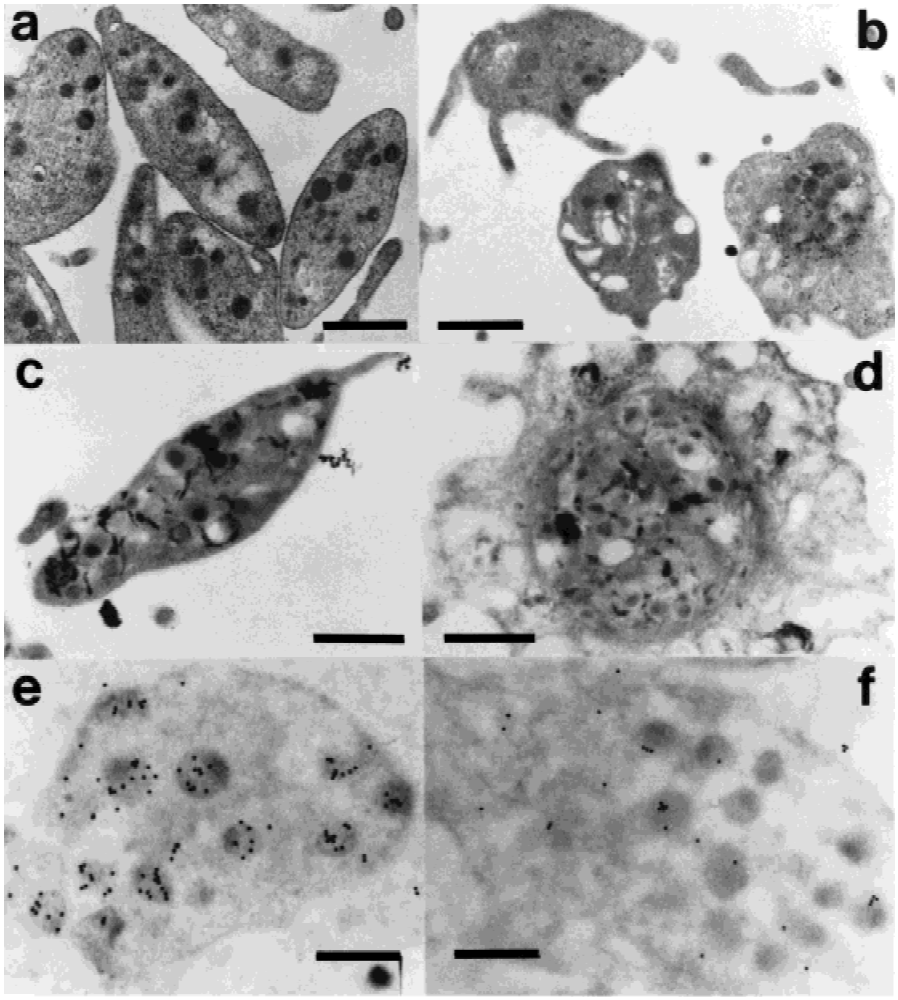

Fig. 1. a: Typical ultrastructure of platelets before bathing. b: Typical ultrastructure of platelets 180 min after the start of bathing. c: Platelet peroxidase reaction was observed in the dark tubular system of a platelet before bathing. d: Weak platelet peroxidase reaction was observed in the dark tubular system of a platelet 180 min after the start of bathing. e: Fibrinogen was localized in α-granules of a platelet before bathing. f: Fibrinogen was decreased in α-granules of a platelet 180 min after the start of bathing. Bars = 1 μm (a–d), 5 μm (e,f).

other plastic tube containing acid citrate dextrose solution and further centrifuged.

Ultrastructural Study

The pellet was used for ultrastructural study by the conventional method under an electron microscope (JEM 200CX, JEOL Ltd., Tokyo, Japan). Two hundred platelets were observed to evaluate the frequencies of fold, pseudopods, vacuoles, and centralization. PPO reaction was performed by the method of Breton-Gorius et al. [8]. Immunoelectron gold staining was performed by the method of Cramer et al. [9]. Anti-fibrinogen polyclonal antibody and goat antimouse immunoglobulins fractions coupled to colloidal gold particles were purchased from Dakopatts (Copenhagen, Denmark) and Sigma (St. Louis, MO), respectively.

The degree of PPO staining and immunogold staining as tentatively expressed as follows. Full staining of PPO or immunogold was scored as 3. Intermediate, weak, and no staining were scored as 2, 1, and 0, respectively. The total score was calculated by counting 100 platelets.

Statistical Analysis

Student's *t*-test was used to evaluate the significance of differences between two categorical variables.

RESULTS

The sublingual temperature showed the highest value 5 min after the start of the 3-min 47°C bath and the mean increase was 1.8°C from 36.8 to 38.6°C. However, the temperature returned to the baseline level within 60 min.

Figure 1a shows typical platelets presenting no morphologic change before bathing. In contrast, platelets with fold, pseudopods, vacuoles, and centralization were observed 15 min after the start of bathing (Fig. 1b). The frequencies of fold, pseudopods, vacuoles, and centralization were increased 15 min after the start of bathing. Although the frequencies of pseudopods and vacuoles were decreased 180 min later, those of fold and centralization were further increased (Fig. 2a). PPO activity was detected only in the dark tubular system before bathing (Fig. 1c), but weaker PPO reaction was scarcely observed

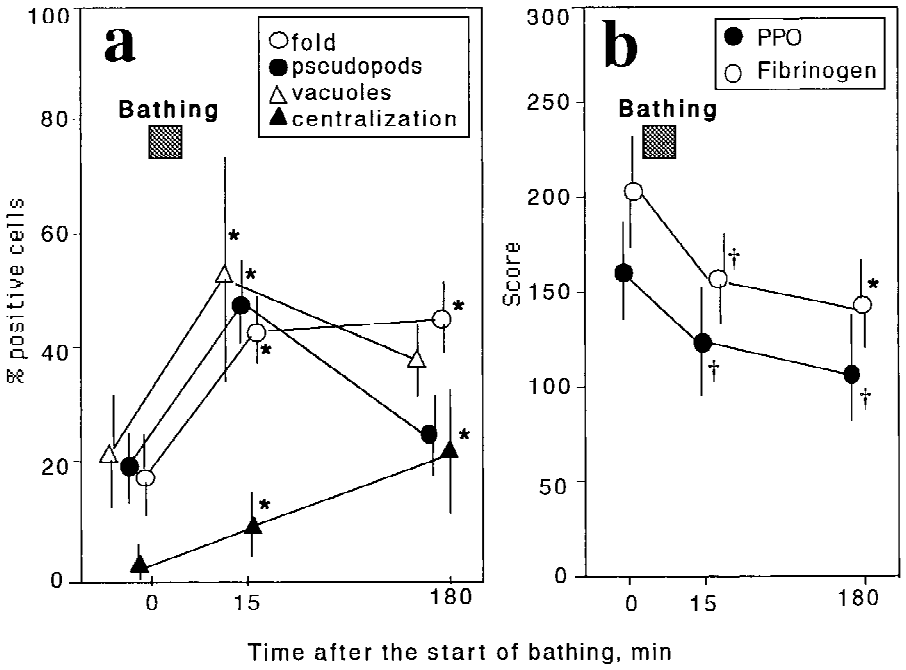

Fig. 2. a: Ultrastructural change in platelets after bathing. Mean ± SD. b: Change in PPO and fibrinogen in platelets after bathing. Mean ± SD. *Significant ($P < 0.01$) compared with the level before bathing. †Significant ($P < 0.05$) compared with the level before bathing.

after bathing (Fig. 1d). Similarly, though fibrinogen was detected exclusively in α-granules before bathing (Fig. 1e), it was decreased in α-granules 15 min after the start of bathing (Fig. 1f). Figure 2b shows the changes in PPO and fibrinogen following a 3-min 47°C bath. Slight but significant decreases in PPO and fibrinogen were recognized after bathing.

DISCUSSION

This study demonstrated morphologic change in platelets along with decreased peroxidase activity and fibrinogen content following hyperthermal stress in vivo. White and Rao et al. reported remarkable platelet shape change by in vitro heating above 42°C and excretion of fibrinogen via the open canalicular system by in vitro heating above 45°C [7,10]. Our results are not inconsistent with their observations, though platelets were activated above 38.6°C in vivo. The difference between in vitro and in vivo platelet activations may be caused partly by direct hyperthermal action of very hot hot-spring bathing on the endothelial cells [6].

We previously reported a preliminary study of CD62 (GMP-140) of platelet after a 15-min incubation at 47°C in vitro [11]. A trend toward increase in mean value of CD62-positive platelet at 47°C was observed though it was not significantly different from the control value. Furthermore, we demonstrated a slight but significant increase in plasma level of β-thromboglobulin but not of platelet factor-4 after in vivo heating, 3-min bathing at 47°C [12]. In addition, the flow cytometric analysis in platelet activation is reported to be less sensitive than the measurement of β-thromboglobulin and platelet factor-4 [13]. Considered together, platelet-activation associated surface markers such as CD62 examined by flow cytometry may not be changed by bathing at 47°C, which increases sublingual temperature by 1.8°C. As a slight increase in β-thromboglobulin was observed after in vivo heating, an increase in CD62 may be expected.

PPO was reported to be detected in the dark tubular system and to catalyze the reaction of prostaglandin synthesis from arachidonic acid [8]. Therefore, decreased PPO activity after hyperthermal stress may be due to the consumption of peroxidase by prostaglandin production. Fibrinogen reserved in α-granules was described to be released to the exterior of platelet through the open canalicular system [9,14,15]. Similarly, decreased fibrinogen in α-granules after hyperthermal stress may result from the secretion of fibrinogen to the platelet exterior.

Thrombogenesis is not evoked solely by platelet activation [16], but is triggered mainly by endothelial injury or crack due to atherosclerosis [17]. However, platelet activation conjointly with decreased fibrinolytic activity and abrupt change in blood pressure and heart rate may cause thrombotic events in a hyperthermal situation.

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